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NIAID Lyme Disease Extramural Research Efforts

Introduction

The NIAID has had a long-standing commitment to conduct research on Lyme borreliosis, or Lyme disease, beginning more than 20 years ago when the cause of the disease was not yet known. In 1981, NIAID-funded research efforts resulted in identifying *Borrelia burgdorferi*, a spiral-shaped bacterium, or spirochete, as the causative agent of Lyme disease (Science 216: 1317, 1982). Since then, basic and clinical research efforts have been expanded in scope to address many different aspects of this infectious disease.

NIAID's mission is to study infectious diseases and host immune defense mechanisms; therefore, the Institute conducts and supports most of the basic and clinical research on Lyme disease. However, because Lyme disease affects different tissue/organ systems of the body, it is a matter of great concern to other NIH institutes and centers as well. The National Institute on Aging (NIA), the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), the National Institute of Mental Health, and the National Institute of Neurological Disorders and Stroke (NINDS) focus on those aspects of Lyme disease that relate to their specific missions. In addition, the Fogarty International Center (FIC) funds research on Lyme disease abroad whereas the National Center for Research Resources (NCRR) provides assistance in allocating resources needed to implement research.

The major goals of the NIAID Lyme Disease Research Program are to develop better means of diagnosing, treating, and preventing this disease. Below is a summary of NIAID-supported extramural research activities supporting these objectives by examining antibiotic therapy, autoimmune reactivity, co-infection, and the development of improved diagnostics and novel vaccines.

Antibiotic Therapy

Early acute Lyme borreliosis is easily cured by conventional antibiotic therapy. However, some patients who have been correctly diagnosed initially as having Lyme disease manifest various neurological and musculoskeletal symptoms several months after receiving what appeared to have been successful antibiotic therapy. Since it is unclear whether such symptoms are due to a long-term persistent infections or other causes, the term post-treatment chronic Lyme disease (PTCLD) is often used to describe this condition, so as not to imply any judgment on the actual mechanism(s) that may be involved.

New England Medical Center (NEMC) Clinical Study

A clinical study on the efficacy of antibiotic therapy for treating PTCLD was completed in late 2000. It was funded through a contract awarded to the New England Medical Center (NEMC) in Boston and involved randomized, double-blind, placebo-controlled multicenter studies to examine the safety and efficacy of ceftriaxone and doxycycline for treating patients with either seropositive or seronegative chronic Lyme disease. The clinical protocols for these studies were developed with extensive review and input from Lyme disease research experts as well as from an NIAID Lyme Disease Advisory Panel composed of patients with Lyme disease, members of patient advocate groups, practicing physicians who treat patients with Lyme disease, and basic research scientists with expertise in either infectious diseases or Lyme disease. The panel provided input on implementing the protocols selected for use in this as well as in intramural clinical studies. Since these clinical studies include an extensive research component, they represent the most

comprehensive and thorough characterization of Lyme disease extant. They have provided—and will continue to generate—a wealth of new information that can be used to devise more effective therapeutic approaches.

On November 14, 2000, the Data and Safety Monitoring Board (DSMB) for the NEMC clinical trials on the efficacy of antibiotic therapy for the treatment of chronic Lyme disease reviewed a planned interim analysis of the data obtained to date. These trials involved testing the safety and efficacy of intensive antibiotic treatment in people with Lyme disease who had developed chronic symptoms, despite earlier treatments with antibiotics. The trials compared treatment with 30 days of intravenous ceftriaxone followed by 60 days of oral doxycycline to treatment with intravenous placebo followed by oral placebo for the same duration, using patients who were either seropositive or seronegative at the time of enrollment. Both trials enrolled patients with a well-documented history of prior Lyme disease and used the same regimen.

After its review, the DSMB unanimously recommended that NIAID terminate the treatment component of these studies since it had accomplished its objectives. Their preliminary analysis showed that after 90 days of continuous antibiotic therapy there were no significant differences in the percentage of patients who felt that their symptoms had improved, gotten worse, or stayed the same between the antibiotic treatment and placebo groups in either trial.

The DSMB's review suggested there was only a slight chance that a difference between the placebo- and antibiotic-treated groups would be found even with continued accrual of another 131 patients, the number needed to reach full enrollment. Therefore, the Board recommended that the treatment component of the studies stop enrolling new patients and that those currently receiving treatment discontinue that therapy. It further recommended that the investigators continue to follow the study patients to monitor their longer-term safety and to obtain additional information that might have value in determining the underlying basis of chronic Lyme disease and in suggesting more effective therapeutic approaches. These rather extensive follow-up studies are still in progress. No new therapeutic studies are contemplated until they have been completed and the results analyzed. The results of the NEMC clinical trials have been published (New Eng J Med 345:85, 2001). They also indicate that patients with PTCLD do not show objective evidence of cognitive impairment, and that 90 days of continuous antibiotic therapy is not more beneficial for these patients than administering placebo (Neurology 60: 1916, 2003).

State University of New York (SUNY) Clinical Study

In another placebo-controlled study conducted at the State University of New York (SUNY) at Stony Brook, 55 patients with PTCLD were given either ceftriaxone or placebo (intravenous) for 28 days. They were then evaluated to determine whether there was significant improvement with respect to fatigue, cognitive function, and the clearance of OspA antigen that was present in the spinal fluid of only 16 percent of all enrolled patients. Ceftriaxone therapy was associated with improvement in fatigue but not with other primary outcome markers considered (Neurology 60:1923, 2003). Because fatigue, which is a non-specific symptom, was the only primary outcome measure affected and because the treatment examined was associated with adverse events, the results of the SUNY study do not support the use of additional antibiotic therapy with parenteral ceftriaxone in post-treatment, persistently fatigued PTCLD patients. We do not know whether the benefit observed for fatigue is due to antibacterial effects or some other mechanism. Additional follow-up studies on patients enrolled in both the NIAID-supported NEMC and SUNY studies revealed that 28 to 90 days of antibiotic therapy had no beneficial effect with respect to several measures of cognitive function (Neurology 60:1916, 2003).

Findings

It is noteworthy that in both the NEMC and SUNY clinical trials cited above, 40 percent and 19 percent, respectively, of patients given placebo alone reported improvement in their symptoms (placebo effect). This underscores the need to conduct placebo-controlled trials to obtain a true and accurate assessment of the beneficial effects of any given therapeutic regimen being

proposed. Otherwise, one would be unable to rule out improvements attributable to the placebo effect. Also, aside from their ability to eliminate persisting infection, several antibiotics have beneficial effects that can alleviate some of the musculoskeletal and neurological symptoms generally associated with chronic Lyme disease. For example, the anti-inflammatory properties of doxycycline and tetracyclines are well-known, and several beta lactam and cephalosporin antibiotics—including ceftriaxone which is commonly used to treat chronic Lyme disease—have neuroprotective properties that can positively influence various neurological disorders (Nature 433: 73, 2005). In view of these considerations, it is clear that valid conclusions on the efficacy of any proposed therapy for treating chronic Lyme disease can be derived only by conducting carefully designed, placebo-controlled clinical trials in which sufficient numbers of patients are enrolled to permit rigorous statistical analysis.

Using another therapeutic approach, an open pilot study was conducted on the efficacy of gabapentin for the symptomatic treatment of chronic neuropathic pain in patients with late-stage Lyme borreliosis. Although only 10 patients were enrolled in this small study in which gabapentin was administered orally for 1-2 years, improvement was noted in both pain quality and pain quantity (9 of 10 patients), as well as a positive effect on mood, general feeling of health, and quality of sleep (5 of 10 patients). These promising initial findings (Dematology 211, 123, 2005) suggest that gabapentin monotherapy may be efficacious in treating pain associated with neuroborreliosis , thereby improving the quality of life for patients with late-stage Lyme borreliosis.

Both the intramural and extramural studies mentioned above involve data collection as well as the maintenance of specimen repositories. Such specimens have been made available to other investigators working on Lyme disease and thus have contributed significantly to the development of improved and/or novel diagnostic procedures.

Animal Models

Appropriate animal models also have provided considerable information on the transmission and pathogenesis of Lyme borreliosis, as well as on the mechanisms involved in the development of protective immunity. NIAID, in collaboration with NINDS, has broadened these efforts to include comprehensive studies on non-human primate animal models for experimental research on the neuropathology associated with chronic Lyme borreliosis (Annals Neurology 56: 361, 2004). A major goal of these studies is to optimize the Rhesus model of Lyme borreliosis as well as to determine the pathogenesis of the disease with a focus on the neurological manifestations. We anticipate these studies will expand our knowledge of those factors that contribute to the pathology associated with persistent infection of the central nervous system by *B. burgdorferi* and ultimately will enable us to devise more effective clinical approaches for treating chronic Lyme borreliosis in humans. They also will supplement and enhance the results of current clinical studies on the efficacy of antibiotic therapies for treating chronic Lyme disease and provide precedents for use in designing future clinical studies. and ultimately enhance the results of current clinical studies on chronic Lyme disease.

Inflammation of skeletal muscle is a consistent feature of Lyme borreliosis, both in humans and in experimental animal models of infection. Although several cytokines are expressed in muscle tissue, proinflammatory cytokines commonly associated with inflammation are not upregulated in *Borrelia*-infected muscle. However, the expression of B-lymphocyte chemoattractant (BLC), a chemokine implicated in the trafficking of B-cells to tissues, is increased in *Borrelia*-infected muscles of non-human promates (Cytokin 19, 297, 2002). Using protein expression profiling, it has been shown that BLC is upregulated in the spinal fluid of patients with neuroborreliosis, but not in patients with noninflammatory and various other inflammatory neurological diseases (Neurology 65, 448, 2005). Since the upregulation of BLC was found in every neuroborreliosis patient examined, it may be a valuable diagnostic marker for neuroborreliosis.

Other studies have shown that *B. burgdorferi* can be detected in mice for at least three months after treatment with therapeutic doses of various antibiotics (ceftriaxone, doxycycline, or azithromycin). These surviving spirochetes could not be transmitted to healthy mice and some

lacked plasmid genes associated with infectivity. By six months, antibiotic-treated mice no longer tested positive for the presence of *B. burgdorferi*, and even cortisone immunosuppression failed to alter this result; that is, it failed to activate infection. Nine months after antibiotic treatment, low levels of *Borrelia* DNA still could be detected in some—but not all—of the mice. These findings (J Infect Dis 186: 1430, 2002) indicate that noninfectious *B. burgdorferi* can persist for a limited period of time after antibiotic therapy. The implications of these findings to persistent infection and the nature of chronic Lyme disease in humans remain to be assessed.

Alzheimer's Disease

Since various published reports suggested the possibility that *B. burgdorferi* may play a role in the etiology of Alzheimer's disease, NIAID intramural scientists examined this issue in greater detail. The results of a recent study, using a very sensitive PCR assay capable of amplifying a Borrelia-specific DNA target sequence from all strains of *B. burgdorferi sensu lato* species known to cause disease in humans, provided no evidence to indicate the presence of Borrelia in the brains of deceased patients with Alzheimer's disease (J Inf Dis 182: 1006, 2000). Although the Lyme Urinary Antigen Test (LUAT) is one of several diagnostic tests used routinely in NIAID's clinical studies on chronic Lyme disease, the results of independent quality control assessments of tests conducted by extramural and intramural scientists showed the LUAT to be unreliable because it yields an unacceptably high percentage of false positive reactions (Amer J Med 110: 217, 2001). By contrast, the similar assessments confirmed a high degree of reproducibility and concordance (virtually 100 percent) for the results obtained using ELISA and Western blot assays (Amer J Med 110: 217, 2001).

Future Research

NIAID expects to sustain a high level of activity in research on Lyme disease in the coming years. It will continue to encourage research on alternative modes of therapy as well as the development of animal models that mimic human infection to enhance or supplement ongoing clinical studies. NIAID investigators conducting controlled clinical studies on therapeutic approaches for treating chronic Lyme disease participated in an NINDS-sponsored conference on neuroborreliosis to facilitate the exchange and application of new knowledge as well as to foster collaborative interactions. Since the complete genome of *B. burgdorfer*i has been sequenced by scientists at The Institute for Genomic Research (Nature 390: 580, 1997), the widespread application of this information will play a significant role in increasing our understanding of the pathogenesis of Lyme disease at the molecular and cellular levels, as well as to accelerate the development of improved diagnostic tests. Researchers already are applying microarray technology to this end.

The Role of Autoimmune Reactivity

The results of recent studies conducted by NIAID and NINDS intramural scientists indicate that T cells from patients with chronic Lyme disease are reactive not only against *B. burgdorferi*-specific antigens but also against various host (self) antigens (Nature Medicine 5: 1375, 1999). Such antigenic mimicry might generate autoimmune inflammatory reactions that could be responsible for arthritic as well as some neurological symptoms associated with chronic Lyme disease. Intramural and extramural scientists are exploring the implications of this finding.

Antibodies against the OspA epitopes of *B. burgdorferi* also have been shown to cross react with neural tissue (J. Peripheral NervousSystem 9, 165, 2004; J Neuroimmunol 159, 192, 2005) as well as myocin (J Clin Microbiol 43, 850, 2005). Such antigenic mimicry may have the potential to generate autoimmune inflammatory reactions that could be responsible for the neurological symptoms associated with chronic Lyme disease. Intramural and extramural scientists are evaluating this possibility in greater detail. In this context, it is interesting to note that homologies

between proteins of *B. burgdorferi* and thyroid antigens also have been reported (Thyroid 14, 964, 2004).

In clinical studies conducted by NIAID-supported extramural scientists, case subject patients with post-treatment chronic Lyme disease (PTCLD) were compared to control subjects without such symptoms for the presence of several human leukocyte antigen (HLA) class II (DRB1 and DQB1) genetic markers, some of which are known to be associated with the expression of autoimmune reactivity. The results obtained did not support the involvement of an autoimmune mechanism in PTCLD (J Infect Dis :192, 1010, 2005) . However, since not all autoimmune diseases are associated with specific HLA haplotypes, these findings do not necessarily exclude that possibility. Definitive proof clearly would involve demonstrating the presence of significant levels of relevant autoimmune antibodies and/or autoreactive T cells in patients with PTCLD but not in treated control subjects without such symptoms. A greater frequency of DRB1*0401, which has been reported to be associated with antibiotic-treatment resistant arthritis (Science 281: 703, 1998) was noted in the case subject patients; although this finding appeared to be nominally significant ($p < 0.05$), its biological significance is ambiguous since none of the case subjects considered had symptoms of inflammatory arthritis.

Co-Infection

Co-infection could represent a major potential problem, mainly because the *Ixodes* ticks that transmit *B. burgdorferi* often carry—and simultaneously transmit—other emerging pathogens such as *Anaplasma (Ehrlichia)* species, the causative agent of human granulocytic ehrlichiosis (HGE), and *Babesia microti*, which causes babesiosis. In Europe and Asia, *Ixodes* ticks also are known to transmit tick-borne encephalitis viruses. Fortunately, this tick-borne viral infection has not yet been reported in the United States, although co-infections with Powasan virus and deer tick virus have been reported.

Co-infection by some or all of these other infectious agents may interfere with the clinical *A.*diagnosis of Lyme borreliosis and/or adversely influence host defense mechanisms, thereby altering landmark characteristics of the disease and the severity of infection (J Infect Dis 186: 428, 2002; J Infect Dis 186, 428, 2002). Studies conducted by NIAID grantees indicate that co-infection with HGE increases the severity of Lyme borreliosis (Infect Immun 69, 3359, 2001). By contrast, when mice were co-infected with *B. microti* and *B. burgdorferi*, neither agent influenced the course of infection induced by the other as evidenced by the percentage of parasitemia, spleen weights, and hematologic and clinical chemistry parameters (J. Infect. Dis. 192, 1634, 2005). Basic research on the pathogenesis and transmission of disease produced by these co-infecting pathogens has increased in recent years, and we are encouraging new grant submissions through Program Announcements designed to expand research on emerging infectious diseases.

In NIAID-supported clinical studies on chronic Lyme disease, patients with persisting symptoms were examined to determine if they might have been co-infected with other tick-borne infectiouis diseases at the time of their acute episode of Lyme disease. Among the tick-borne infectious diseases considered were babesioisis (*Babesia microti*), granulocytic ehrlichiosis (*Anaplasma phagocytophilia*), and tick-borne encephalitis virus infection. The seroprevalence rates for *B. microti* and *A. phagocytophilia* were found to be 2.5 percent and 8.6 percent, respectively and no patient examined was found to be positive for tick-borne encephalitis viruses (Vector Borne Zoonotic Dis 2: 255, 2002). Thus, the persistence of symptoms in patients with "post-Lyme syndrome" could not be attributed to co-infection with one of these pathogens.

An examination of pathogen distributions in the tissues of mice infected with both *B. burgdorferi* and *A. phagocytophilium*, the bacterium that cause HGE in humans, showed an increase in the numbers of *B. burgdorferi* in the ears, heart base, and skin of co-infected mice; however, the numbers of *A. phagocytophilium* remained relatively constant. The serum antibody response to *A. phagocytophilium* – but not to *B. burgdorferi* – decreased as a result of co-infection. These findings suggest that co-infection can influence not only pathogen burden but also host antibody responses (Infect Immun 73: 3440, 2005).

NIAID intramural and extramural research programs have initiated clinical studies on chronic Lyme disease. The intramural research program is conducting a comprehensive clinical, microbiological, and immunological assessment of patients with Lyme disease. This involves multiple lines of investigation with emphasis on:

- Defining various biological markers of infection
- Assessing clinical course and outcomes of patients with Lyme borreliosis
- Characterizing the immune response generated in response to *B. burgdorferi*

Diagnostic Procedures

NIAID is supporting various efforts to evaluate and improve existing diagnostic procedures. Approximately 20 percent of its extramural Lyme disease research portfolio is devoted to developing novel and more sensitive diagnostic procedures. New applications are submitted for review and funded on a regular basis. In addition to significant efforts in the area of diagnosis, NIAID grantees work directly with CDC scientists to evaluate and compare the effectiveness of currently used diagnostic methods.

In collaboration with CDC, NIAID also plays a major role in encouraging the development of novel approaches to improve the diagnosis of Lyme borreliosis in humans with various co-infections (e.g., ehrlichiosis or babesiosis), as well as in immunized people. For example, NIAID grantees have shown that a synthetic peptide composed of 26 amino acid residues (C6) derived from a variable surface antigen (VlsE) of *B. burgdorferi* can be used in a new, rapid, and extremely sensitive ELISA test (the C6 ELISA) for diagnosing Lyme disease. Because this diagnostic test for Lyme disease, which has been approved by FDA, does not detect antibodies specific for recombinant OspA, it can be used even for those who have been immunized with the licensed OspA-based LYMErix vaccine (J Clin Microbiol 40: 2591, 2002).

Although the Lyme Urinary Antigen Test (LUAT) is one of several diagnostic tests used routinely in NIAID's clinical studies on chronic Lyme disease, the results of independent quality control assessments of tests conducted by extramural and intramural scientists showed the LUAT to be unreliable because it yields an unacceptably high percentage of false positive reactions (Amer J Med 110: 217, 2001). A critical evaluation of urine-based PCR assays for the diagnosis of Lyme borreliosis likewise affirmed that urine is not a suitable material for the diagnosis of Lyme borreliosis (Clin. Diag. Lab. Immunol. 12, 910, 2005). By contrast, the similar assessments confirmed a high degree of reproducibility and concordance (virtually 100 percent) for the results obtained using ELISA and Western blot assays (Amer J Med 110: 217, 2001).

Of great importance is the fact that decreases in the titer of antibodies against C6 can be used as an indicator of the efficacy of antibiotic therapy for patients with localized or disseminated Lyme disease, but not for chronic Lyme disease (Eur J. Clin Microbiol Infect Dis 23: 615, 2004). This is indeed a major advance since no other laboratory test enables one to obtain such information (J Clin Microbiol 40: 2591, 2002). The results obtained with the C6 ELISA are consistent with those obtained with other diagnostic tests and may obviate the time and expense for conducting additional laboratory tests to confirm the diagnosis of Lyme disease (Clin Diag Lab Immunol 11: 924, 2004). The NIAID- supported investigators are now working closely with the CDC to determine if the C6 ELISA can eventually replace the traditional two-tiered conventional ELISA and Western blot assays. The results of other studies confirmed that a decline in the anti-C6 antibody titer coincides with the efficacy of antimicrobial therapy in patients with early localized or early disseminated Lyme borreliosis (Clin Diag Lab Immunol 12: 1069, 2005).

The *Borrelia burgdorferi*-specific immune complex (IC) test, in which polyethylene glycol (PEG) is used to isolate ICs from serum, has been advocated by some investigators as an approach for the early diagnosis of active borreliosis. However, recent findings indicate that it may not be more effective in detecting early and active infections than other conventional tests in which unprocessed serum specimens are used (Clin Diag Lab Immunol 12: 1036, 2005).

There is a great need to develop additional simple, sensitive, and rapid procedures to distinguish those who are actively infected with *B. burgdorferi* from those who have either recovered from a previous infection or have been immunized previously. Since the genome of *B. burgdorferi* has now been completely sequenced, greater advances towards this goal are anticipated as this information is used in conjunction with microarray technology and proteomics to improve diagnosis as well as provide new insights on the pathogenesis of this disease and pathogen-specific host response mechanisms.

Transmission of Lyme Disease

Researchers do not completely understand the molecular basis of how *B. burgdorferi* maintains itself in nature via a complex life cycle that involves passage through ticks and various intermediate hosts, such as mice and deer, before infecting humans. The outer surface protein A (OspA) of *B. burgdorferi* has been well studied, and there is much speculation about its role — in conjunction with other cell surface proteins (OspB and OspC) — in transmitting Lyme disease (J Clin Invest 113: 1093, 2004).

Although *B. burgdorferi* depends on *Ixodes* ticks and mammalian (rodent) hosts for its persistence in nature (J Clin Microbiol 38: 382, 2000), the search for borrelial genes responsible for its parasitic dependence on these types of diverse hosts has been hampered by limitations in the ability to genetically manipulate virulent strains of Borrelia. Despite this constraint, there is evidence to indicate that the inactivation and complementation of a gene (BBE16) encoded by a linear plasmid (lp25) plays a major role in the virulence, pathogenesis, and survival of *B. burgdorferi* during its natural life cycle (Mol Microbiol 48: 753, 2003). This gene, which has been renamed BptA (for borrelial persistence in ticks-gene A), potentiates virulence in mice and is essential for the persistence of *B. burgdorferi* in *Ixodes scapularis* ticks. Although BptA appears to be a lipoprotein expressed on the outer surface membrane of *B. burgdorferi*, the molecular mechanism(s) by which BptA promotes persistence within its tick vector remains to be elucidated. Since BptA appears to be highly conserved (>88 percent similarity and >74 percent identity in amino acid sequence) in all *B. burgdorferi sensu lato* strains examined, it may be widely used to promote persistence in nature. Given the absolute dependence on — and intimate association with — its tick and rodent hosts, BptA must be considered to be a major virulence factor that is critical for *B. burgdorferi's* overall infectious strategy (Proc Natl Acad Sci 102: 6972, 2005). Strategies designed to block the synthesis or expression of BptA could be of great value in preventing the transmission of Lyme disease.

Given the potential role that differentially up-regulated surface proteins play in the transmission of borreliosis and Lyme disease pathogenesis, other investigators have conducted a comprehensive gene expression profiling analysis of temperature-shifted and mammalian host-adapted *B. burgdorferi*. The combined microarray analyses revealed that many genes encoding known and putative outer surface proteins are down-regulated in mammalian host-adapted *B. burgdorferi*. However, at the same time, several different genes encoding at least seven putative outer surface proteins were found to be up-regulated during the transmission and infection process. All seven are immunogenic and generate the production of bactericidal antibodies in infected baboons (Infect Imm 74, 296, 2006). This suggests that these outer surface proteins might be excellent second-generation vaccine candidates.

The above findings are consistent with the results of other published studies (J Infect Dis 186:1430, 2002) in which a novel experimental technique (xenodiagnosis by ticks) was used to determine whether *B. burgdorferi* can persist in mice long after antibiotic therapy. Here, an immunoflourescence assay and the polymerase chain reaction (PCR) assay were used to demonstrate that *B. burgdorferi* could be detected in doxycycline- and ceftriaxone-treated mice for at least three months—if not longer—after antibiotic therapy. However, the resulting surviving spirochetes are unable to infect other naive mice because they lack those linear plasmids (lp25 and lp28) that are essential for their ability to transmit infection (J Infect Dis 186:1430, 2002). It is noteworthy that lp25 also encodes for a gene product (PncA or BBE22) that is essential for the survival of *B. burgdorferi* in a mammalian host (Mol Microbiol 48:753, 2003).

NIAID-supported investigators have now been able to create various mutant strains of *B. burgdorferi* and show that although OspA and OspB are not required for infection of mice, they are essential for the colonization and survival of *B. burgdorferi* in ticks. *Ixodes scapularis* ticks have a receptor on the inner wall of their intestines to which *B. burgdorferi* are able to bind tenaciously by means of OspA, a cell surface protein. This receptor is called the “tick receptor for OspA” or TROSPA. Attachment to TROSPA enables *B. burgdorferi* to persist in the gut from the time they are ingested by ticks through a subsequent molt, thereby avoiding elimination; this enables Borrelia to be injected into a new host when ticks take their next blood meal (Cell 119: 457, 2004). When ticks take a blood meal, the production of OspA is down-regulated in favor of the increased production of OspC. This causes gut-bound spirochetes to become detached,

which enables ticks then to migrate to the salivary glands where they can be injected into mammalian hosts. Thus, TROSPA in addition to other bacterial cell surface components such as OspA appear to play a key role in the transmission of Lyme disease to humans. Other studies have shown that if ticks are permitted to feed on mice that have been immunized previously with OspA, or have been treated with antibody specific for OspA, the attachment and subsequent colonization of ticks by *B. burgdorferi* is significantly impaired if not prevented. This suggests the feasibilty of developing oral or vector expressed transmission-blocking vaccines, that involve the immunization of intermediate hosts upon which ticks feed (Proc Natl Acad Sci 101: 18159, 2004). Several NIAID-supported investigators are now examining and testing this approach under controlled, laboratory conditions.

Other studies conducted by NIAID-supported investigators (Nature 436: 573, 2005) demonstrate that *B. burgdorferi* utilizes an immunosuppressive tick salivary protein (Salp 15) to facilitate the transmission of infection to mammalian hosts. This is based on observations that: (a) the level of Salp 15 expression is enhanced by the presence of *B. burgdorferi* in infected ticks; (b) Salp 15 adheres specifically to spirochete surface OspC both *in vivo* and *in vitro*, thereby increasing the ability of *B. burgdorferi* to infect mice; and (c) the binding of Salp 15 protects *B. burgdorferi* from antibody-mediated killing in vitro, a factor that confers marked survival advantage. All of these findings suggest that Salp 15 and/or other tick salivary proteins might be excellent candidates for vaccines to block the transmission of Lyme disease (Parasitol 129: S161, 2004). In this context, prior and repeated exposure of experimental animals to uninfected ticks -- and presumably their salivary proteins-- has been shown to limit the capacity of infected ticks to transmit Lyme disease (J Emerg Infect Dis 11: 36, 2005).

Vaccine Production

Two large pharmaceutical companies have devoted considerable effort to developing a vaccine for Lyme disease. Double-blind, randomized, placebo-controlled clinical trials, involving more than 10,000 volunteers from areas of the United States where Lyme disease is highly endemic, have been completed for each of two *B. burgdorferi* recombinant OspA vaccines manufactured by GlaxoSmithKline (formerly SmithKline Beecham [SKB]) and Pasteur Merieux Connaught (PMC). These vaccines were found to be 49 to 68 percent effective in preventing Lyme disease after two injections, and 68 to 92 percent effective in preventing Lyme disease after three injections. The duration of the protective immunity generated in response to the SKB vaccine (LYMErix), which was licensed by FDA in December 1998, is not known. Consequently, the need for yearly booster injections remains to be established. Researchers and health experts anticipate the use of these vaccines in endemic areas will likely result in significantly reducing the incidence of Lyme disease in the future.

NIAID was not directly involved in the design and implementation of these particular vaccine trials; however, patents for cloning the genes used for the expression of recombinant OspA, as well as knowledge of the role of antibodies against OspA in the development of protective immunity, were derived from basic research grants funded by NIAID.

In April 2002, GlaxoSmithKline announced that even with the incidence of Lyme disease continuing to rise, sales for LYMErix declined from about 1.5 million doses in 1999 to a projected 10,000 doses in 2002. Although studies conducted by FDA failed to reveal that any reported adverse events were vaccine-associated, GlaxoSmithKline discontinued manufacturing the vaccine for economic reasons (Vaccine 20:1603, 2002).

NIAID-funded investigators have developed an experimental bait delivery system for an OspA-based vaccine against *B. burgdorferi* in which mice are immunized orally (via gavage or bait feeding) with a strain of *Escherichia coli* expressing the gene for OspA. This results in the appearance of serum antibody specific for OspA. Upon exposure to *Ixodes* nymphs carrying multiple strains of *B. burgdorferi,* oral vaccination was found to protect 89% of the mice from infection and the resultant serum antibody response confirmed the presence of IgG2a/2b antibody specific for OspA. This vaccination approach is able to generate a significant protective

immune response against a variety of infectious strains of *B. burgdorferi,* thereby indicating that it can eliminate *B. burgdorferi* from a major host reservoir. It suggests that the broad delivery of an oral vaccine to wildlife reservoirs in an endemic area is likely to disrupt the transmission of Lyme disease (Vaccine, *in press)*. These findings are consistent with the results reported by other investigators (Proc Natl Acad Sci 52: 18159, 2004), thereby affirming the utility of this approach.

In other studies, NIAID grantees have developed a murine-targeted OspA vaccine utilizing Vaccinia virus to interrupt the transmission of disease in reservoir hosts, thereby having the potential to reduce the incidence of human disease. Oral vaccination of mice with a single dose of Vaccinia expressing OspA resulted in high antibody titers to OspA, 100 percent protection of vaccinated mice from infection by *B. burgdorferi*, and a significant clearance of *B. burgdorferi* from infected ticks fed on vaccinated animals (Vaccine 24: 1949, 2006). These findings indicate that such a vaccine may effectively reduce the incidence of Lyme disease in endemic areas. Field studies of this vaccine are planned.

NIAID also is funding preclinical studies on developing and testing other candidate vaccines (for example, decorin-binding protein A or DbpA) for Lyme disease. MedImmune, Inc. (an NIAID Small Business Innovation Research grantee) and Sanofi-Aventis Pharmaceuticals, reported that a combination vaccine composed of the DbpA and OspA of *B. burgdorferi* is more effective than either given alone in preventing the development of borreliosis in experimental animals. On the basis of these encouraging findings, both companies have entered into an agreement to develop a new, more effective second-generation vaccine to prevent Lyme disease in humans. Although the results of previous studies indicate that DbpA induces the development of protective immunity in a murine model of Lyme borreliosis when mice are challenged (needle inoculated) intradermally with in vitro-cultivated *B. burgdorferi*, such mice are not protected from infection transmitted by ticks carrying virulent *B. burgdorferi*.